Supporting information

Anatomical and chemical responses of eastern white pine (*Pinus strobus* L.) to blue-stain (*Ophiostoma minus*) inoculation

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Table of Contents

LC-ESI-PDA-MS^n operating conditions 4

LC-PDA operating conditions 4

Supplemental figures 6

Supplemental tables 11

Figure S1. Standard calibration curves for phenolic compound quantitation. 6

Figure S2. Spectrum of each compound considered for analysis from RSLC – PDA. 8

Figure S3. Calibration curves used for *P. strobus* monoterpene quantification. 9

Figure S4. Comparison of *p*-coumaric acid for control and *O. minus* inoculated trees at 28- and 65- days post inoculation (dpi). Bars represent the standard error of the mean. Control N=2 and inoculated N=4-6. 10

Figure S5. Comparison of taxifolin hexoside and procyanidin dimer (B-type) for control and *O. minus* inoculated trees at 28- and 65- days post inoculation (dpi). Inoculation is marginally insignificant ($p = 0.06$) for taxifolin hexoside but significant for procyanidin dimer (B-type) ($p = 0.03$). 10

Table S1. Chromatographic, mass spectral, photodiode array and putative identities of phenolic compounds obtained from phloem tissue of *P. strobus*. 11

Table S2. Statistical analysis to model the effect of treatment and collection time on each compound. For taxifolin hexoside and procyanidin B-type we used response variable absolute amount (nmol g^{-1} FW). For resveratrol-O-glucoside thru Unk4, we used peak area as a response variable ($p < 0.05$ highlighted in bold). *p*-Coumaric acid did not have enough replicates for the control trees therefore no statistical analysis was performed. 12

Table S3. Non-parametric Kruskal-Wallis test for Epi/catechin comparing control versus *O. minus* inoculated trees. 13

Table S4. Non-parametric Kruskal-Wallis test for Epi/catechin comparing days post inoculation. 13

Table S5. Effect of *O. minus* inoculation on β-pinene in *P. strobus*. 13

LC-ESI-PDA-MS^n operating conditions

Operating conditions for the LC-ESI-MS were developed and optimized in house and was loosely based upon (Villari et al., 2012). The separation was carried out using a 150 mm Thermo Fisher Scientific Accucore (C18) column (particle size 2.6 µm, I.D 4.6 mm) (Thermo Fisher Scientific, Waltham, MA) on a Thermo Fisher Ultimate 3000 series system. The mobile phase consisted of 0.1% acetic acid in water (solvent A), and 0.1% acetic acid in methanol (Solvent B), with a flow rate of 1 ml min^{-1}. The following linear gradient (cumulative run time (min), flow rate (ml min^{-1}), % solvent A) was used: 0.0, 1, 95.0; 0.5, 1.0, 95.0; 2.0, 1.0, 25.0; 6.5, 1.0, 33.0; 8.5, 1.0, 40.0; 9.0, 1.0, 50; 9.5, 1.0, 100.0; 13.0, 1.0, 100.0 (total run time 13 min). A 2 µl plant extract sample was injected and analyzed by PDA and LC-MS^n (in serial mode). A post-column splitting "T" was fitted to divide the LC eluent to divert majority of the eluate to waste. A 450 µL min^{-1} eluent was allowed to flow through the H-ESI (heated electro spray ionization) source and Orbitrap MS. Ions in the MS were introduced by employing a negative ion ESI mode with a negative ion spray voltage of 3000 V, ion transfer tube and vaporizer temperatures of 333 °C and 300 °C respectively.

Gas flow was as follows, sheath gas (arb) 40, aux gas (arb), and sweep gas (arb) 1. 12 of −80 V. In the initial screening, survey scan was set to m/z 50 – 1,000. The most intense ion was fragmented to MS^2 and subsequently to MS^3 and MS^4. A dynamic exclusion filter was applied to avoid ions that are appearing frequently (if the ion appears in two consecutive scans, they were avoided for 60 s). CID collision energy was maintained at a 35% level and the ions were held inside the trap for 10 ms. The Xcalibur (ver 2.0, Thermo Fisher Scientific, Waltham, MA) allowed data dependent tandem MS generation. Data acquisition and subsequent processing were performed using Xcalibur software.

LC-PDA operating conditions

Rapid separation liquid chromatography (RSLC) - PDA was carried out using an identical instrument with an identical column. The Chromeleon (Ver. 7.2, Thermo Fisher Scientific, Waltham, MA) was utilized for data acquisition and further analysis. Instrument operating conditions were exactly the same as above except the solvents (A) and (B) contained 2% acetic acid in water and methanol respectively.

The auto-sampler and column temperature were held at 4 °C and 40 °C respectively for all analyses. A 2 µl plant extract from a single sample was injected each time. The PDA detector was set to acquire all the spectra between 220 and 390 nm. Automated chromatographic output at 280 nm absorbance were extracted simultaneously for phenolic compounds. Peaks from MS1 were overlaid on PDA trace, manually aligned and identified based on standards retention time, mass spectral fragmentation, PDA match and using existing literature. Results are presented in figures S1, S3 and Table S1 below.

Supplemental figures

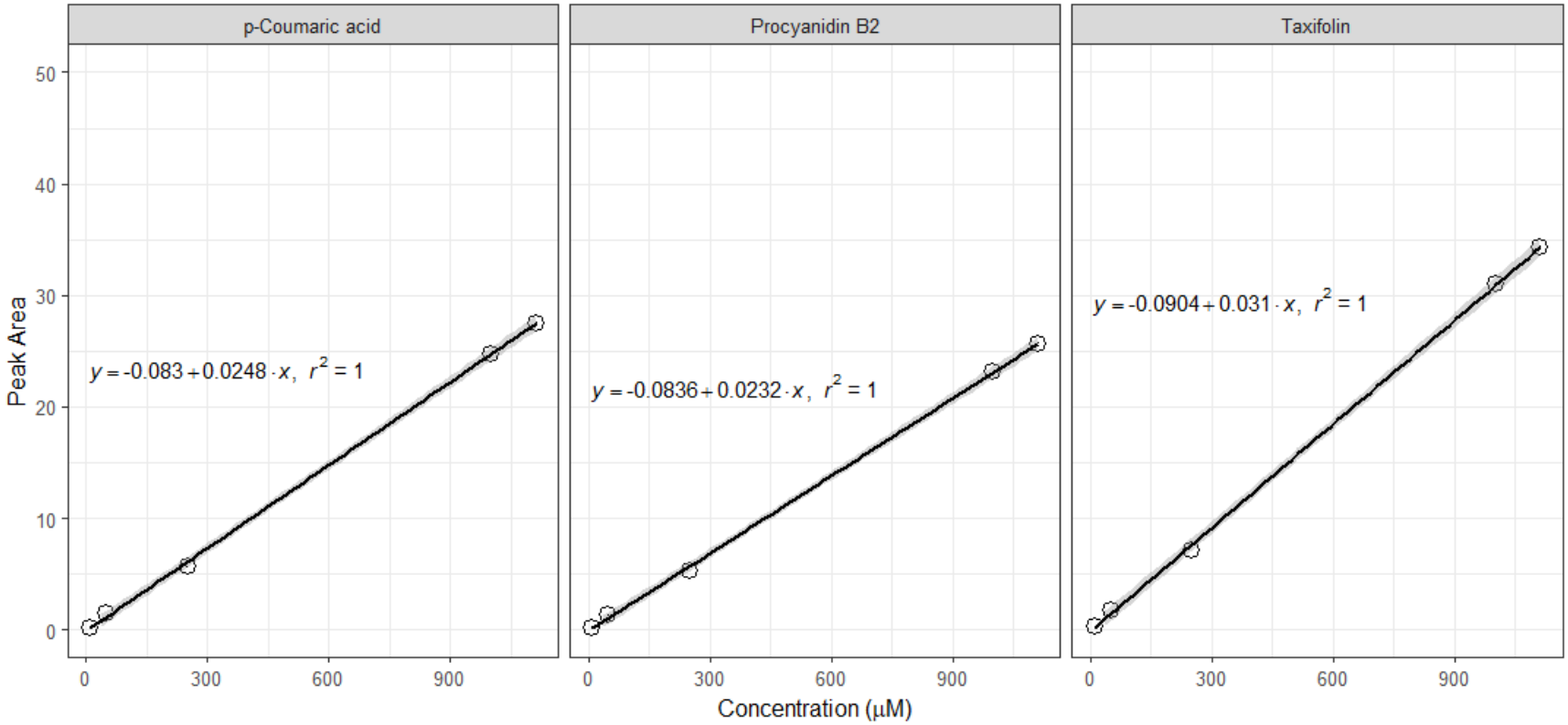
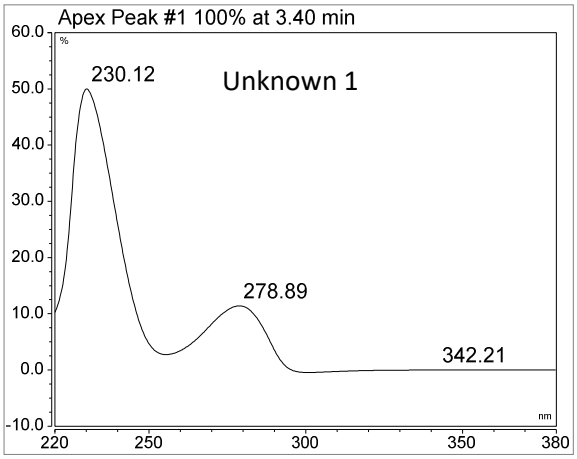


Figure S1. Standard calibration curves for phenolic compound quantitation.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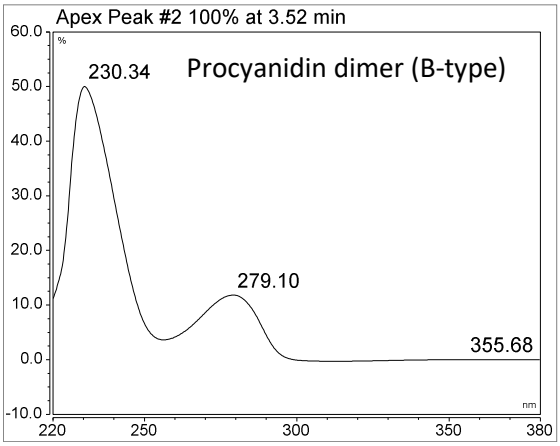
278.89
342.21


Apex Peak #2 100% at 3.52 min
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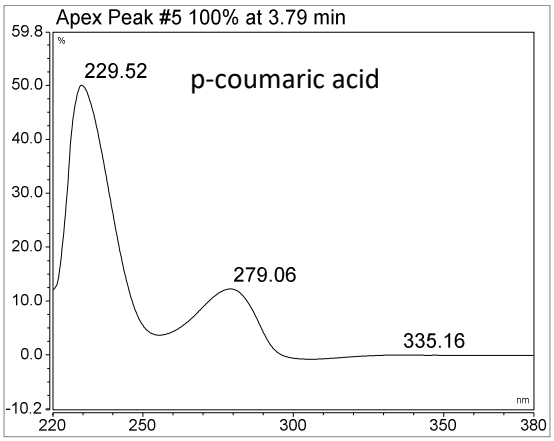
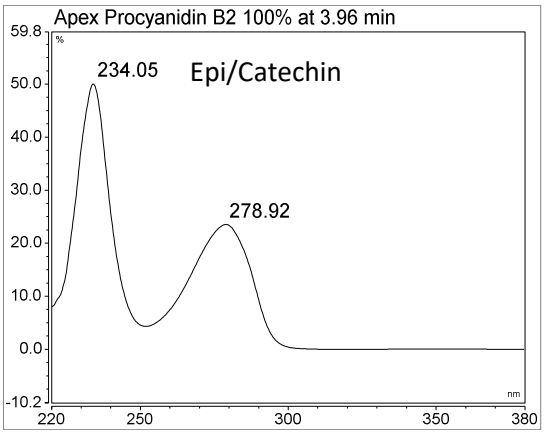
Procyanidin dimer (B-type)
279.10
355.68


Apex Peak #5 100% at 3.79 min
229.52
p-coumaric acid
279.06
335.16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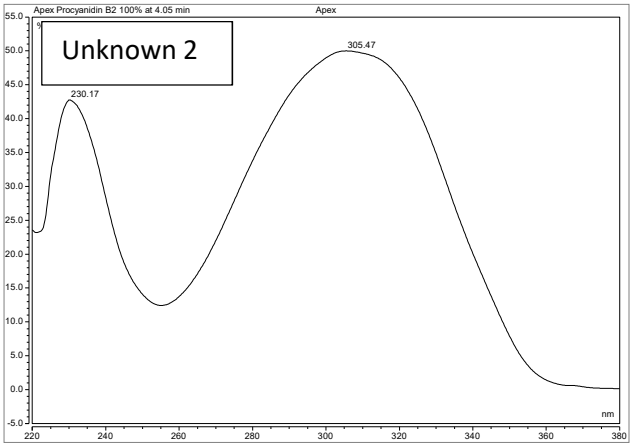
Epi/Catechin
278.92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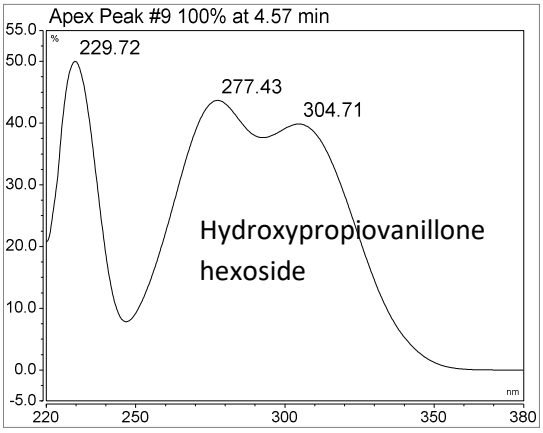
305.47


Apex Peak #9 100% at 4.57 min
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277.43
304.71
Hydroxypropiovanillone hexoside

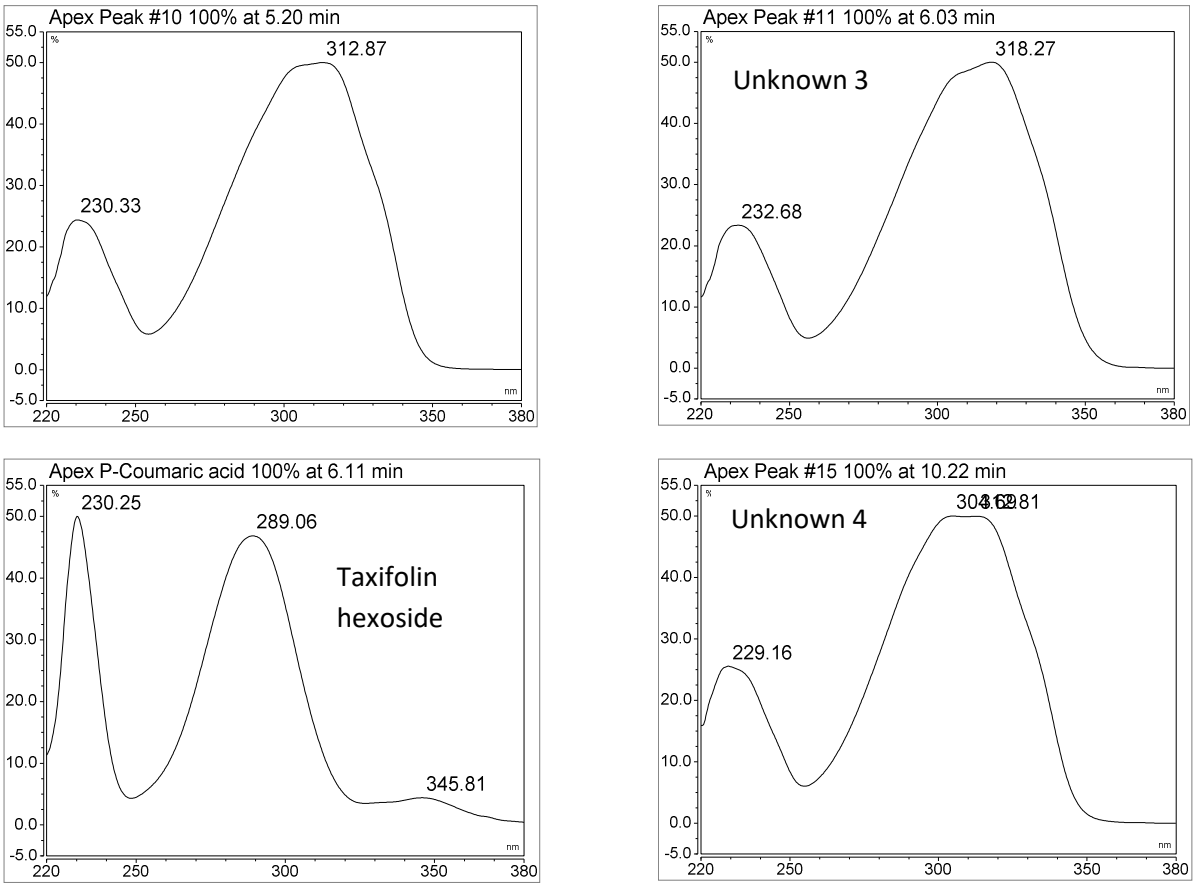

Figure S2. Spectrum of each compound considered for analysis from RSLC – PDA.

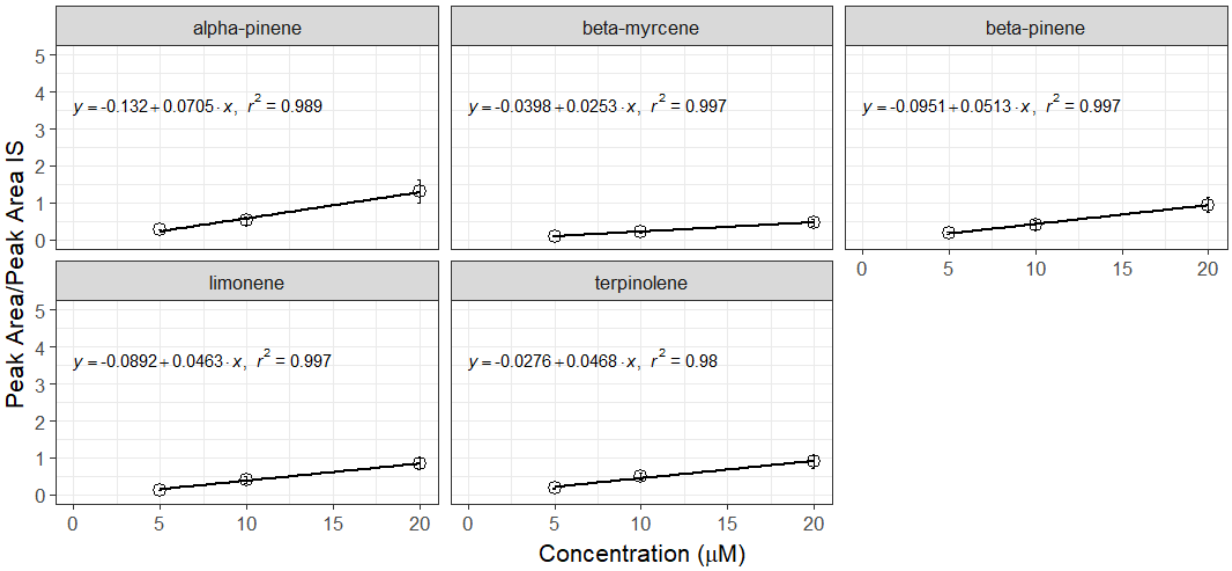

Figure S3. Calibration curves used for *P. strobus* monoterpene quantification.

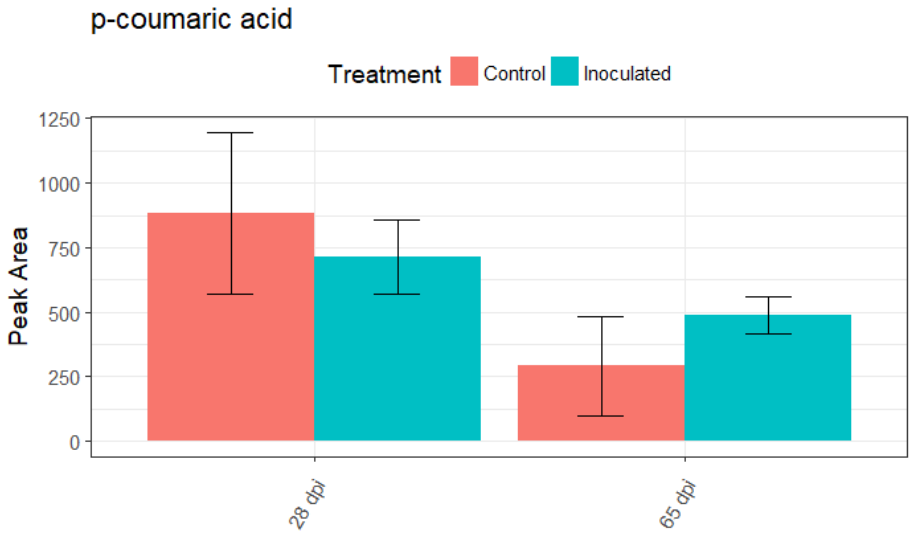

Figure S4. Comparison of *p*-coumaric acid for control and *O. minus* inoculated trees at 28- and 65- days post inoculation (dpi). Bars represent the standard error of the mean. Control N=2 and inoculated N=4-6.

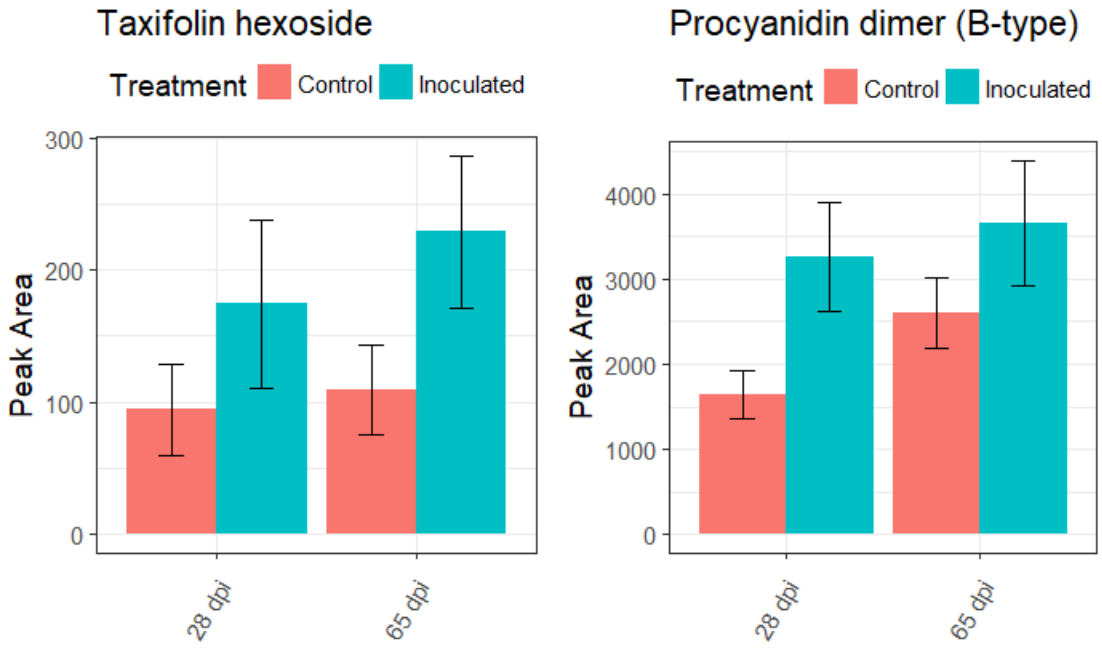

Figure S5. Comparison of taxifolin hexoside and procyanidin dimer (B-type) for control and *O. minus* inoculated trees at 28- and 65- days post inoculation (dpi). Inoculation is marginally insignificant (p = 0.06) for taxifolin hexoside but significant for procyanidin dimer (B-type) (p = 0.03).

Supplemental tables

Table S1. Chromatographic, mass spectral, photodiode array and putative identities of phenolic compounds obtained from phloem tissue of *P. strobus.*

Peak Number	Retention Time	$[M-H]^-$	MS2	MS3	MS4	λ_{max} (nm)	Identity	Standard Equivalent	Reference
1	3.40	403.141	240.963	225.013	181.099	279	Unknown 1	-	
2	3.52	577.137	425.122	407.023	285.011	279	Procyanidin dimer (B Type)[a]	Procyanidin B2	Karonen et al 2004a Villari et al 2012
3	3.79	163.040	118.971	66.38		285	p-coumaric acid	p-coumaric acid	Verified by standard
4	3.96	289.013	245.124	203.012		280	Epi/Catechin	-	Karonen et al 2004b Villari et al 2012
5	4.05	401.146	341.018	179.011		283	Unknown 2	-	
6	4.56	357.120	177.053	162.031		277 sh.304	Hydroxypropiovanillone hexoside	-	Karonen et al 2004a Wallis et al 2011 Villari et al 2012
7	5.19	389.126	227.115	184.966	143.014	304	Resveratrol-O-glucoside	-	Fu et al 2015
8	6.04	425.102	389.138	227.000	185.048	307 sh.317	Unknown 3	-	
9	6.14	501.082	465.032	447.091		289	Taxifolin Hexoside	Taxifolin	Karonen et al 2004a Wallis et al 2011 Villari et al 2012
10	10.22	403.141	241.062	225.04	181.009	305	Unknown 4	-	

Sh: Shoulder

a: Minimal coelution towards the tail with an unknown compound

Table S2. Statistical analysis to model the effect of treatment and collection time on each compound. For taxifolin hexoside and procyanidin B-type we used response variable absolute amount (nmol g^{-1} FW). For resveratrol-O-glucoside thru Unk4, we used peak area as a response variable (p <0.05 highlighted in bold). *p*-Coumaric acid did not have enough replicates for the control trees therefore no statistical analysis was performed.

	Phenolic metabolites															
	Taxifolin hexoside		Procyanidin dimer B-type		Resveratrol-O-glucoside		Hydroxy--propiovanillone hexoside		Unk1		Unk2		Unk3		Unk4	
Treatments	$F_{1, 17}$	*P-value*	$F_{1, 17}$	*P-value*	$F_{1, 17}$	*P-value*	$F_{1, 16}$	*P-value*	$F_{1, 15}$	*P-value*	$F_{1, 17}$	*P-value*	$F_{1, 16}$	*P-value*	$F_{1, 17}$	*P-value*
Treatment (fungal inoculation)	4.094	**0.059**	5.627	**0.030**	2.531	0.130	0.186	0.672	10.980	**0.005**	6.254	**0.0236**	0.566	0.463	9.167	**0.008**
Collection times	0.497	0.490	1.350	0.261	0.019	0.892	0.837	0.374	0.163	0.692	4.992	**0.0401**	2.667	0.122	10.900	**0.004**
Treatment × Collection times	0.152	0.702	0.239	0.631	0.162	0.692	0.048	0.830	1.464	0.245	4.906	**0.0416**	0.448	0.513	0.016	0.901

Table S3. Non-parametric Kruskal-Wallis test for Epi/catechin comparing control versus *O. minus* inoculated trees.

Chisq	Df	p.chisq
6.662	1	0.009847

Table S4. Non-parametric Kruskal-Wallis test for Epi/catechin comparing days post inoculation.

Chisq	Df	p.chisq
0.2842	1	0.594

Table S5. Effect of *O. minus* inoculation on β-pinene in *P. strobus.*

Chisq	Df	p.chisq
9.524	1	0.002028